

Biodegradation of Coproducts from Industrially Processed Corn in a Compost Environment¹

Syed H. Imam^{2,3,4} and Sherald H. Gordon²

Information pertaining to biodegradability of renewable polymeric material is critical for the design and development of single-use biodegradable consumer products. The rate and extent of biodegradation of corn fiber, corn zein, cornstarch, distillers grain, and corn gluten meal were evaluated in compost environments under variable temperature, pH, and moisture conditions. Generally, composts with higher temperature (40°C), neutral pH (7.0), and 50%–60% moisture appeared to be ideal for corn coproduct biodegradation, particularly for corn gluten meal and corn zein. Low moisture conditions slowed biodegradation considerably, but degradation rates improved when moisture content increased up to 60%. Thereafter, increased moisture particularly slowed the degradation of corn gluten meal and corn zein, whereas cornstarch degradation remained unaffected. At low pH (4.0) and high pH (11.0) the rate of degradation of most coproducts was slowed somewhat. Cornstarch degradation was slower at pH 7.0, but degradation improved with increased temperatures. Increase in compost temperature from 25 to 40°C (in 5°C increments) also improved biodegradation of corn fiber and distillers grain. Addition of 1% urea to compost as a nitrogen source decreased the extent of biodegradation nearly 40% for corn gluten meal and corn zein, and 20% for cornstarch samples. Treatment of compost with 0.02% azide inhibited biodegradation of all coproducts, suggesting that the presence of metabolically active microbial cells is required for effective degradation of biobased materials in a compost environment.

KEY WORDS: Biodegradation; compost; corn coproducts; industrially processed corn.

INTRODUCTION

New and/or expanded markets are needed for farm commodities, particularly grains. Agribusiness in the United States faces ever-increasing foreign challenges in

a highly competitive marketplace, while recent U.S. trade imbalances escalate yearly. This, coupled with the growing realization that natural resources are dwindling and pollution is of increasing public concern, make it imperative that we learn how to convert renewable agricultural commodities into higher-value products. Most efforts to enhance the value of commodity crops are generally restricted to surplus crops such as corn and soybeans. However, with increasing world population, falling trade barriers, and diminishing farmland, many such crops will cease to be in surplus. Fortunately, there exists another,

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² Plant Polymer Research, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, 1815 N. University Street, Peoria, Illinois 61604.

³ To whom correspondence should be addressed. Tel: (309) 681-6335; Fax: (309) 681 6691; E-mail: IMAMSH@NCAUR.USDA.GOV

⁴ Current address: Bioproduct Chemistry and Engineering Research, USDA, ARS, WRRRC, 800 Buchanan St., Albany, CA 44710. E-mail: simam@pw.usda.gov

yet untapped, vast agricultural resource that has been overlooked and which can offer far more challenging and unlimited opportunities for use [1, 2]. These are leftovers and residues of crops, such as straw, husk, stalk, peel, pulp, fiber, as well as waste from industrially processed cereal grains such as corn, wheat, and soybean that can be further used by conversion into high-value products.

At present, commercial application of these materials is somewhat limited, but the potential is beginning to be realized [3–5]. A large portion of corn produced in the United States and in some industrialized countries is processed and refined for use in fuel ethanol production and as an additive in animal feed. Many coproducts and residues of corn generated during these processes are low-cost polymeric materials available in enormous quantities. These materials represent complex biological macromolecules such as carbohydrates, proteins, and fibers that are structural biopolymers with secondary and tertiary structures, and offer great potential to replace petroleum-based polymers in future biobased material development strategies.

In particular, plant-based materials have been blended with synthetic as well as renewable polymers to produce biodegradable plastic films [6–9], molded articles [10], and packaging foams [11]. Use of plant materials in bioplastics offers potential for replacing petrochemical-based disposable plastics. The potential degradable nature of such bioplastics is also expected to provide solutions to environmental issues associated with disposal of conventional plastics. Use of corn fibers (CF) and fuel ethanol residues for the production of a sugar substitute, xylitol [12], and complex carbohydrate polymers such as alternan and pullulan [13, 14] have been reported.

Corn-derived ethanol coproducts have been successfully used in developing composite films [15], and in production of commercial fish feed [16], whereas derivatized corn zein (CZ) protein has been shown to chelate metal ions, indicating a possible application in environmental clean-up of heavy metals [17]. Furthermore, the development of biodegradable plastic from CZ protein [18] and polyurethane foams from corn carbohydrates [19] have also been reported by some investigators.

Being inherently biodegradable in nature, these plant-based materials can be ideal raw materials for single-use biodegradable consumer products. However, their successful use in product development and subsequent disposal would require knowledge and understanding of their biodegradation properties, particularly under variable environmental conditions. This report describes the biodegradation of various corn coproducts in compost environments under variable temperatures, pH values,

and moisture conditions, as well as in compost supplemented with 1% urea as a nitrogen source. This study provides useful information pertaining to environmental factors involved in the biodegradation of corn coproducts in compost to predict their functional lives and environmental residence times.

EXPERIMENTAL

Samples

Corn coproducts including CF, CZ, cornstarch (CS), corn gluten meal (CGM), and distillers grain (DG) were received free of charge from the Williams Bioenergy, Inc., Pekin, IL. These materials were produced at their facility during the wet milling and fuel ethanol production from corn.

Composition

Carbon and nitrogen contents in samples were determined using the automatic model 2000 carbon/hydrogen/nitrogen (CHN) analyzer (LECO Corporation, St. Joseph, MI). Percent ash and moisture content in samples were measured using the protocols 08-01 and 44-15A, respectively, listed in the Methods of the American Association of Cereal Chemists, Inc., 9th edn., St. Paul, MN. Protein was measured using the Kjeldahl method.

Biodegradation Studies

About 0.5 g of each sample of known carbon content was mixed with 25 g of compost (Garden Basics Topsoil, Swiss Farm Products, Las Vegas, NV) under appropriate conditions in a 250-mL sample chamber of a fully computerized, closed-circuit Micro-Oxymax Respirometer System (Columbus Instruments, Inc., Columbus, OH) equipped with an expansion interface, a condenser, and a water bath. The sample chamber was placed in a water bath controlled at 25°C and connected to the Micro-Oxymax Respirometer. With some exceptions, most moisture and temperature experiments were carried out over a period of 13 days. The pH experiments were carried out over 20 days. CO₂ evolution from each sample in quadruple was measured every 6 hr. The CO₂ measurements of samples were averaged, and had <2% variability. Samples were always accompanied by at least duplicate blanks (compost alone) to correct for background CO₂. Experiments were conducted to study the effects of variable temperature, pH, and moisture regimes on the biodegradation properties of corn coproducts. Typically, temperature of 25°C, pH 7.0, between 50% and

Table I. Composition of Coproducts Recovered from Industrial Processing of Corn^a

	% Moisture	% Ash	% Nitrogen	% Protein	% Carbon
Corn fiber ^b	7.70	0.56	1.91	11.90	45.1
Corn zein ^c	6.33	1.41	14.9	93.10	51.9
Cornstarch ^b	10.50	0.09	0.01	0.06	40.1
Corn gluten meal ^b	7.24	2.29	11.40	71.25	48.9
Distillers grain ^d	6.65	1.41	4.50	28.12	48.9

^aValues in the table represent an average of triplicate samples having <5% variability.

^bCorn fiber, cornstarch, and corn gluten meal are coproducts of wet milling of corn.

^cZein is a specialty product produced industrially by chemical extraction of gluten meal.

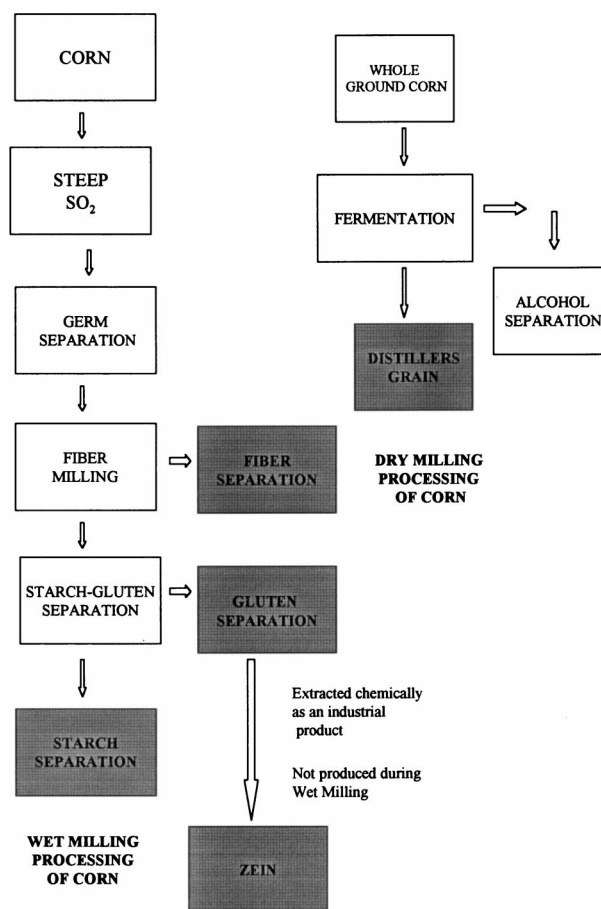
^dDistillers grain, a coproduct of dry milling, is a residue recovered from alcohol production of whole ground corn.

60% moisture content, and a 12-hr day/night light cycle were the optimal conditions under which materials were evaluated for biodegradability. In studies where one condition was varied, the other two conditions remained optimal.

For pH studies, the compost was equilibrated at 50% concentration (w/w) in buffer of desired pH containing 50 mM each of sodium citrate, KHPO₄, and tris-glycine (Sigma Chemical Co., St. Louis, MO). The compost was equilibrated for 4 days until the pH was stabilized. To obtain composts of desired moisture content, deionized water was either added to the compost or the compost was dried at room temperature until the desired moisture content was achieved. Moisture content was monitored with a Sartorius MA 30 moisture analyzer.

Determination of Percent Biodegradation

The total available carbon in the starting material (0.5 g) of each sample was calculated from the predetermined percent carbon values listed in Table I. The values for the total accumulated CO₂ volume during biodegradation for a sample in a reaction chamber were obtained through respirometry. These volume (*V*) data were used in the equation of state of the ideal gas law ($PV = nRT$) to determine the moles (*n*) of carbon converted into CO₂ as a result of microbial processes in a compost, where the molar gas constant $R = 0.082055 \text{ Latm/Kmol}$; $T = 298 \text{ Kelvin (25°C)}$, and pressure $P = 1 \text{ atm}$. The percent biodegradation was obtained from the sum of net moles of CO₂ released in a sample chamber divided by the moles of CO₂ present in the original sample and multiplied by 100.

**Fig. 1.** Flow chart of coproducts recovered from corn processing and ethanol fermentation.

RESULTS

Coproducts

Coproducts recovered during corn processing are roughly outlined in the flow chart (Fig. 1). After the steeping of corn, CFs are separated from the germ, whereas CS and corn gluten are separated from the remaining mixture in the starch–gluten fraction. Most of the CZ protein is found in the gluten fraction, which is chemically extracted as an industrial product. DG comprises the leftover residues from whole ground corn (dry milling) that have undergone fermentation to produce alcohol. Being rich in both partially digested carbohydrates and protein from yeast cells, this coproduct is used primarily as an additive in animal feed. The DG is generally added to corn gluten to produce corn gluten meal (CGM), a more balanced and protein-rich animal feed.

Material Composition

The composition of corn coproducts is provided in Table I. The percent carbon in samples ranged between 40.1 and 51.9. CZ had the highest carbon content and CS had the lowest. CGM and DG both have 48.9% carbon, whereas CF had 45.1% carbon. Expectedly, CZ was the most protein-rich fraction with 93.10% protein content, followed by CGM with more than 71% protein. DG had 28.12% protein content. CF and CS contained the lowest amount of protein, 11.91% and 0.06%, respectively. A similar trend was observed with respect to nitrogen content in samples. All samples had roughly 6%–7% moisture contents, with the exception of CS, which contained close to 11% moisture. Ash contents ranged between 0.56% for CF and 2.29% for CGM.

Biodegradation Studies: Variable Moisture Conditions

About 50%–60% moisture content appeared to be the best condition for effective degradation of corn coproducts in compost (Fig. 2c, d). A marked decrease in both rate and the extent of degradation was observed in samples exposed at either low- or high-moisture conditions. At 30% moisture (Fig. 2a), generally all samples exhibited a longer lag phase extending up to 4–6 days and degraded slowly. At the end of 13 days exposure, only 12%–38% degradation was reached. The highest degradation was observed for CGM (38%) followed by DG (30%), CF (20%), CZ (18%), and CS (12%). When the moisture content increased in increments from 30% to 60%, both the rate and extent of degradation increased rapidly for all samples (Fig. 2a, b, c). Particularly, at 50% and 60% moisture (the moisture range typical of compost), CGM and CZ showed 100% degradation, whereas CS degraded between 60% and 70% in approximately 7–8 days. The CF and DG exhibited only 35%–55% degradation over the same period of time. Note that under 70% moisture conditions, the extent of degradation of CGM, CZ, and CS decreased about 20%, 30%, and 15%, respectively, and their rate of degradation slowed, whereas the rate of CS degradation remained unaffected (Fig. 2d).

pH Variations

At neutral pH (7.0) both the rate and extent of degradation for CGM and CZ increased rapidly, reaching 90%–100% degradation, whereas CS, CF, and DG only degraded between 30% and 40% (Fig. 3b). Both acidic (pH 4.0) and alkaline (pH 11.0) compost conditions nega-

tively impacted degradation of CGM and CZ (Fig. 3a, c). Surprisingly, starch degradation, which was only 40% at neutral pH, increased to 60% under both acidic and alkaline pH conditions. Under acidic conditions, CGM and CZ exhibited 30% and 40% less degradation, respectively, within a 20-day time period. However, degradation of CF and DG under acidic conditions was not decreased (Fig. 3a). Under alkaline (pH 11.0) conditions, degradation of CGM and CZ was significantly decreased in both the rate and extent of degradation (Fig. 3c). CGM, CZ, and CS degraded to almost 60%, whereas CF and DG degradation was only 30%. In all samples, there was an initial lag phase of a couple of days, and the degradation rate increased rapidly for the next 5–6 days. Thereafter, overall degradation proceeded slowly. The microbes and their hydrolytic enzymes preferentially degraded CGM and CZ in compost under neutral pH conditions. The slowest degradation was observed in CF and DG samples, and the most extensive degradation of these materials (40%–45%) was possible only under acidic conditions. CS degraded much more rapidly at acidic and alkaline pH, but the extent of degradation was much less at pH 7.0. Addition of 0.02% azide, a respiratory inhibitor in compost, markedly inhibited the overall rate and extent of degradation for all samples (only CF, CGM, and DG were tested) (Fig. 4). The CGM degradation was below 40%, whereas degradation of both CF and DG was less than 20% after 20 days. The effect of azide on biodegradation of coproducts was somewhat similar to that of alkaline conditions.

Temperature Variations

Generally, increasing the compost temperature from 25°C to 40°C (in 5°C increments) considerably shortened the initial lag phase and enhanced degradation of all samples including CF and DG (Fig. 5a, b, c). Typically, under normal experimental conditions, both CF and DG degraded at a much slower rate and degradation rarely exceeded 40%. In the compost maintained at 40°C, however, the degradation of these samples almost doubled within 13 days. The CS degradation was consistently between 70% and 80% in all temperature regimes tested.

Addition of 1% urea in compost (25°C, pH 7.0, and 50%–60% moisture) as a nitrogen source decreased the extent of biodegradation about 40% for both CGM and CZ (Fig. 6), whereas the CS samples exhibited a marked increase in the rate of degradation. Initially, there was almost no lag phase, and CS degraded at a rapid rate for 6–8 days (7.5%/day). The biodegradation curve for CS seems to suggest a biphasic activity.

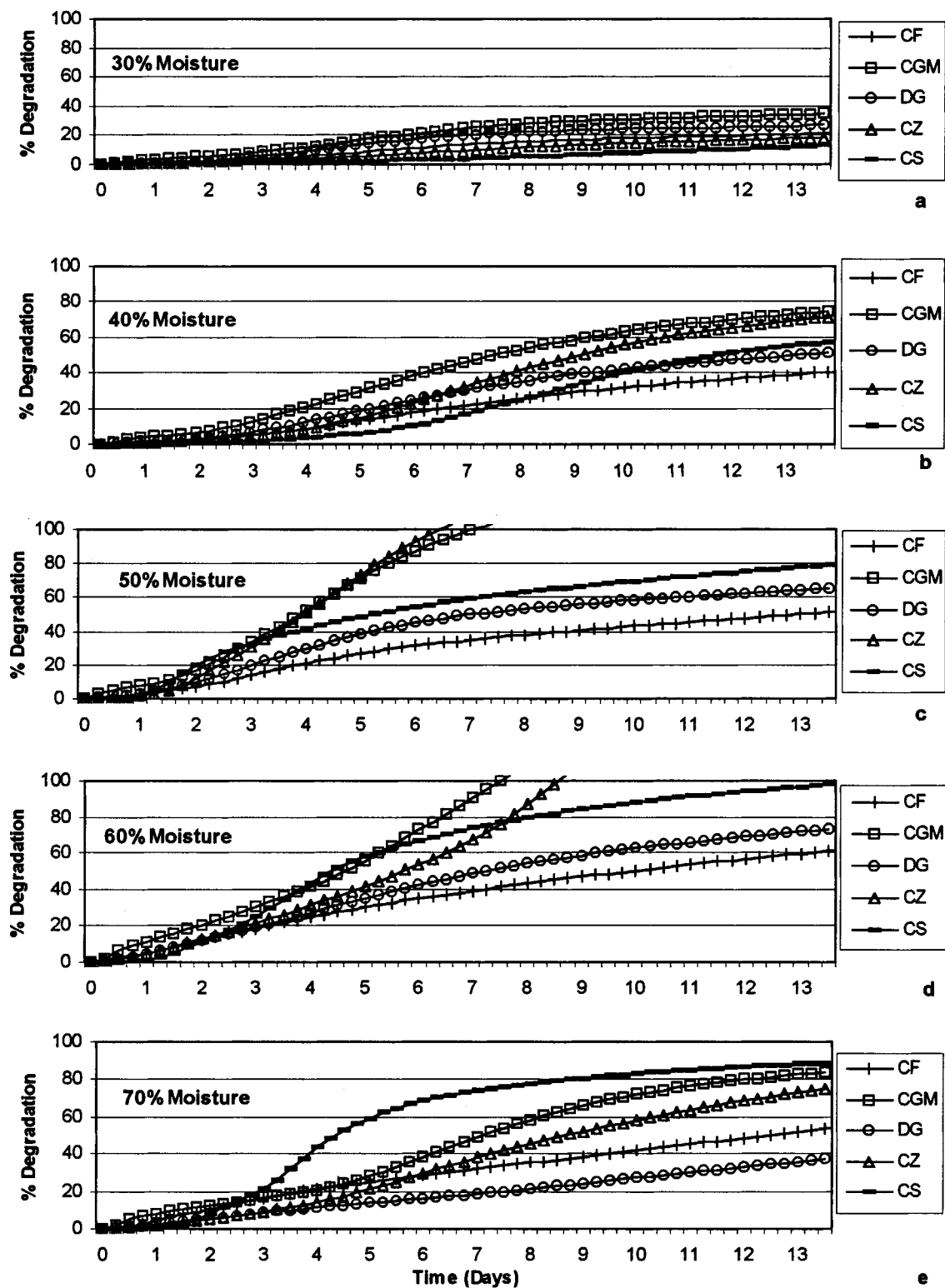


Fig. 2. Biodegradation of coproducts in compost (pH 7.0, 25°C) over 13 days at varied moisture content.

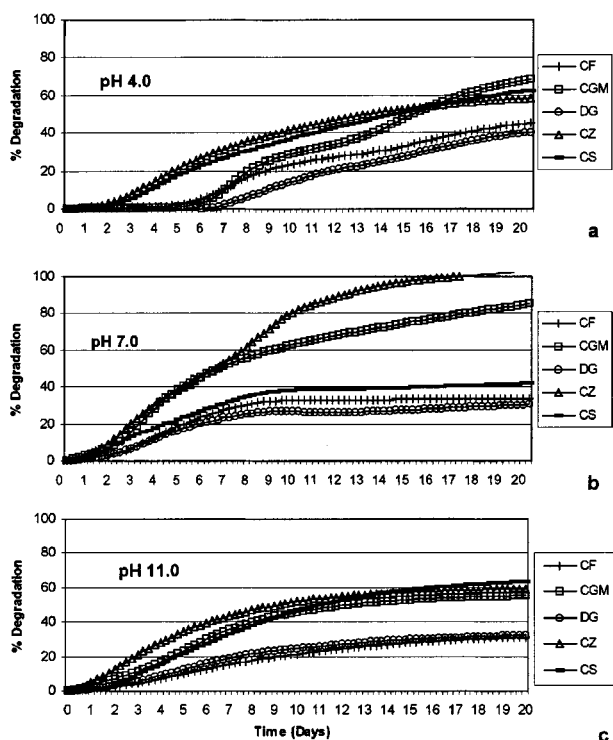


Fig. 3. Biodegradation of coproducts in compost (50% moisture, 25°C) over 20 days under acidic, neutral, and alkaline pH conditions.

DISCUSSION

High-moisture conditions (50%–60% moisture content) apparently provide a more suitable environment for microbial growth and proliferation by causing cells to secrete hydrolytic enzymes. In this regard, it has been shown that high water contents around soil bacteria and cellulolytic fungi, *Phanerochaete chrysosporium*, contribute to cell-wall hydrolysis causing extracellular materials, including hydrolytic enzymes, to leak out of the cells [20, 21]. The apparent differences in the rate and

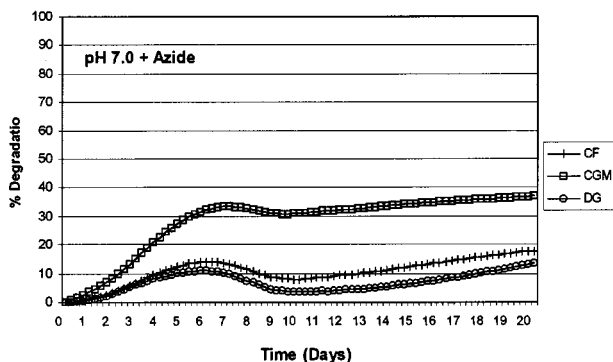


Fig. 4. Biodegradation of CGM, DG, and CF over 20 days in compost (pH 7.0, 25°C, and 50% moisture) to which 0.02% azide was added.

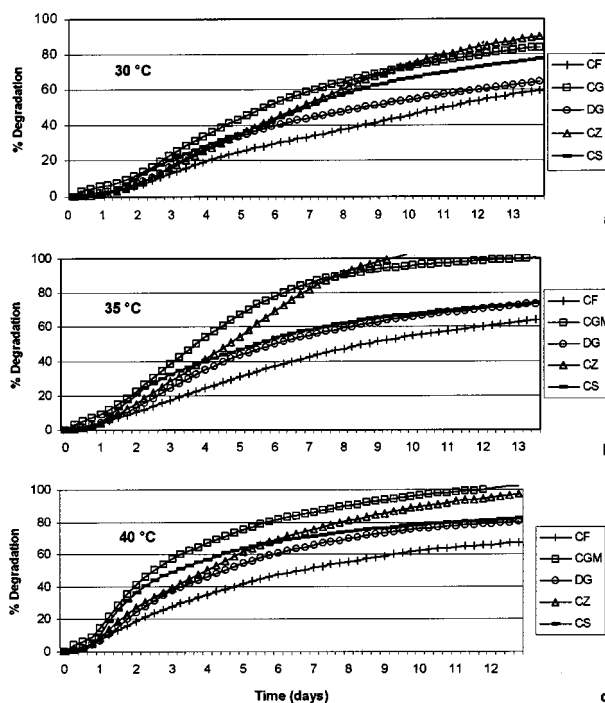


Fig. 5. Biodegradation of coproducts in compost (pH 7.0, 50% moisture) over 13 days at varied temperatures.

extent of biodegradation may be related to the material composition of each sample. For example, CGM, which is a mixture of DGs and CZ-extracted gluten, rich in soluble sugars and partially digested carbohydrates and proteins, offers both an excellent carbon source for microbial growth and a substrate for enzymatic hydrolysis. In contrast, CF, which contained mostly cellulose, a hydrophilic and a highly crystalline complex carbohydrate polymer known for its slow enzymatic hydrolysis, degraded slowly. Because of their slow biodegradability in natural environments, cellulosic matrices are also being used to encapsulate active agents in applications where

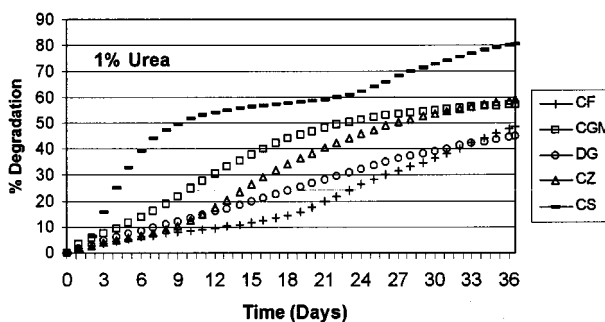


Fig. 6. Biodegradation of coproducts in compost (pH 7.0, 25°C, and 50% moisture) over 36 days in the presence of 1% urea (dry weight basis).

sustained release of an active agent is highly desirable [22, 23].

Microbial enzyme activities are sensitive to the pH of the compost. Enzymes require optimal temperature and pH conditions for best performance. Enzymes that degraded CGM and CZ preferentially required neutral (pH 7.0) conditions, and either acidic (pH 4.0) or alkaline (pH 11.0) conditions significantly impacted their catalytic capacity, resulting in lower degradation rates. Enzymatic hydrolysis of many polymers was achieved at pH 7.0. In this regard, it has been reported that the molar size of the enzymatically extracted polysaccharides from rye meal decreased proportionally with decrease in pH [24]. Only plant cell wall polysaccharide-degrading enzymes and glycosidases in rumens are known to perform catalysis of plant materials at pH as low as 2.0 [25]. Conversely, some hemicellulases, including xylanases, are known to be active at higher pH (>8.0) and high temperature (60°C); these are desirable enzyme properties for delignification in the paper industry [26]. The CF and DG apparently contained mostly cellulosic material more resistant to hydrolytic enzymes and both of these materials degraded no more than 40% under all pH conditions. Marked decrease in degradation rates caused by the presence of the respiratory inhibitor (0.02% azide) indicated that involvement of metabolically active microorganisms was required for effective biodegradation of corn coproducts in compost.

Increased temperatures generally improved the biodegradation of corn coproducts. In particular, CF and DG degradation were enhanced considerably when the compost temperature was increased to 40°C. Most commercial composts are produced following the American Composting Council's guidelines, which require that compost mature at elevated temperatures (50–60°C). Environmental microbes that survive this treatment produce secretory depolymerases, which are apparently capable of working more effectively under thermophilic conditions. Enzymes produced by soil microorganisms such as lignin-degrading enzymes used in kraft pulp bleaching [27] as well as hemicellulases and xylanases [26] used in paper industry work best at temperatures well over 50°C. Carbohydrate-degrading enzyme activities of xylanases and beta-glucanases from the commonly found soil yeast *Trichoderma reesei* have been shown to increase with temperature [24].

Also, activity of starch degrading α -amylase produced by *Bacillus licheniformis* was found to be stable at temperatures as high as 90°C [28]. A study conducted to evaluate the environmental decomposition of lignocellulosic wastes indicated that changes in ratios of elements (C, N, H, and O) in compost were observed both at low

and high compost temperatures [29]. This indicated that microbes in a stabilized compost environment, depending on the environmental conditions and the available carbon source, may selectively produce enzyme(s) capable of functioning under given conditions. Nitrogen is a limiting factor in soil. Addition of urea as a nitrogen source to a compost has been shown to improve the compostability of biological materials [30]. Compared to its effect on cellulosic fibers and proteins, urea probably was more effective in interrupting inter- and intra-molecular hydrogen bonding in CS, as well as providing a nitrogen source for microbial growth which led to improved degradation of CS in compost.

Eventually, successful biodegradation of biological materials must depend on microbial catalysis as well as environmental parameters. Environmental factors such as water, oxygen, redox potential, nutrients, pH, and temperature all in some manner could influence enzyme catalysis of plant polymers. However, to fully understand and evaluate the biodegradation capacity of soil, compost, or water environments, it is essential that we seek and acquire knowledge pertaining to the factors that control the desired metabolic capacity, availability of microbes in a stable environment, and their hydrolytic enzymes, as well as the type of carbon sources available in a given environment. Investigation of these factors is continuing from this work.

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